

CHAPTER

13

General Laboratory Principles, Quality Assessment, and Safety

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I. GENERAL LABORATORY PRINCIPLES

A. Chemicals and Related Substances

1. Chemicals

- a. **Analytic reagent (AR) grade** chemicals meet the specifications established by the **American Chemical Society (ACS)** and are used in most analytical laboratory procedures.
- b. **Ultrapure** reagents have undergone additional processing that makes them suitable for special procedures such as atomic absorption, chromatography, molecular diagnostics, etc.

2. Reagent grade water meets the specifications of **Clinical Laboratory Reagent Water (CLRW)**, the standards for which were established by the Clinical and Laboratory Standards Institute (CLSI). **Reagent grade water (CLRW)** is of the highest quality and is used in test methodologies where minimum interference and maximum precision and accuracy are needed. Resistivity of greater than or equal to $10 \text{ M}\Omega\cdot\text{cm}$ at 25°C is required. Other specifications address microbiological content, silicate, particulate matter, and organics.

- a. Processes required in preparation of reagent grade water (CLRW):
 - 1) **Prefilters** are glass or cotton microfibers that remove 98% of the particulate matter.
 - 2) **Activated carbon** removes organic matter and chlorine.
 - 3) **A submicron filter** removes all particles or microorganisms larger than the membrane pore size.
 - 4) **Reverse osmosis** is a process that removes 95–99% of bacteria and organic and other particulate matter.
 - 5) **Ion exchange** is a system of resin cartridges or tanks connected in series that remove cations and anions to make deionized water.
- b. Other types of water used in the clinical laboratory are categorized by the **intended purpose** of their use and include:
 - 1) **Special reagent water (SRW):** May require different preparation than CLRW according to intended use, such as sterility specification for tissue or organ culture, nucleic acid content for DNA testing, metal content for trace metal analysis, etc.
 - 2) **Instrument feed water:** Used for internal instrument rinsing, making dilutions, etc., and needs to meet manufacturer's specifications.
 - 3) **Water for use as a diluent or reagent supplied by manufacturer:** Label states intended use; do not substitute for CLRW or SRW unless label indicates it is of such quality.
 - 4) **Purified water commercially bottled:** Exercise care because some plastic containers permit microorganism growth due to air permeability.
 - 5) **Water for laboratory dishwashers and autoclaves:** Purified to contain only low levels of organics, inorganics, and particulate matter so it does not leave residue on glassware or contaminate solutions and media in autoclaves.

3. Standards

- a. **Primary standards** are highly purified chemicals that are weighed or measured to produce a solution with an exact concentration.
 - b. **Secondary standards** are solutions whose values are determined by repeated analyses, using a reference method.
 - c. **National Institute of Standards and Technology (NIST)** provides standard reference materials for purchase.
 - d. **Standard reference materials (SRMs) and certified reference materials (CRMs)** are produced by the NIST. Values of the materials are determined by high-quality analysis, and the chemical composition is certified.
4. Units of measure: **Système Internationale d'Unités** is a system of measurement that is known as **SI units**. SI units may be classified as base, derived, or supplemental units. **Base units** were established for each of the **seven fundamental quantities of measurement**.
5. **Desiccants** are **drying agents** that absorb water from air or other materials. **Magnesium perchlorate** is one of the most effective desiccants, and **silica gel** is one of the least hygroscopic. **Desiccators** provide a dry environment for chemical materials.

B. Laboratory Supplies and Equipment

1. Laboratory supplies such as pipettes, flasks, etc. used for analytical work must meet specific **tolerances of accuracy** as designated by the NIST. **Class A** is the designation used when laboratory supplies **meet NIST specifications**.
2. **Types of glass**
 - a. **Flint glass** is made from soda-lime glass. It is inexpensive and used in making some disposable laboratory glassware.
 - b. **Borosilicate glass** is commonly used for laboratory glassware because of its properties, including resistance to heat, corrosion, and thermal shock.
 - c. **Pyrex** and **Kimax** are glasses that can withstand high temperatures. They are made of borosilicate, which has a low alkali content.
 - d. **Corex** is alumina-silicate glass that is six times stronger than borosilicate glass. It is used to make high-temperature thermometers, graduated cylinders, and centrifuge tubes.
 - e. **Vycor** glass can be heated to 900°C and is used for extremely high temperatures and resists heat shock.
3. **Types of plasticware**
 - a. **Polyolefins** (polyethylene/polypropylene): Chemically inert resins; generally resistant to acids, alkalis, and salt solutions
 - b. **Polycarbonate** has a clear appearance and because of its strength may be used for centrifuge tubes. Chemical resistance is less than the polyolefins.
 - c. **Fluorocarbon resins** (Teflon): Chemically inert and used for temperatures from -270 to +255°C; provide nonwetable surface; used for stir bars and tubing

4. Pipettes and calibration

- a. **Transfer pipettes** are volumetric and Ostwald-Folin.
 - 1) **Volumetric pipettes** are “**to deliver**” (TD) types that have the bulb closer to the center and accurately deliver a fixed volume of aqueous solution. They drain by gravity and should not be blown out.
 - 2) **Ostwald-Folin pipettes** are TD types that have the bulb closer to the delivery tip because they deliver viscous fluids. These pipettes deliver an accurate volume by being “blown out” using a pipetting bulb. An **etched ring** or a **pair of etched rings** near the top of the pipette indicates the need to “**blow out.**”
- b. **Measuring pipettes** are serologic and Mohr.
 - 1) **Serologic pipettes** are TD types that are calibrated to the tip and must be “**blown out**” to deliver entire volume. The need to blow out is indicated by the etched rings at the top of the pipette.
 - 2) **Mohr pipettes** are TD types that are calibrated between marks and cannot be “blown out.”
- c. Pipettes labeled “**to contain**” (TC) must be **rinsed out** to deliver the entire contents. Sahli pipettes and some capillary pipettes are in this category.
- d. Semiautomatic and automatic pipettes are handheld and automated, respectively.
 - 1) **Semiautomatic** are handheld pipettes that use disposable tips, and the laboratorian performs aspiration and dispensing.
 - 2) **Automatic pipettes** are electronic and may not require tips. Many use a glass syringe that aspirates and dispenses through the same tube.
- e. Verification of pipette calibration
 - 1) **Gravimetric pipette calibration:** This method verifies the amount of liquid dispensed by a pipette. All equipment and water must be at room temperature before beginning. A specific amount of water is pipetted into a weighed container and the weight of the water is determined. The weight of the water is proportional to the volume of water pipetted.
 - 2) **Volumetric pipette calibration:** This method uses a dye of known concentration and water. A specific amount of dye is pipetted into a specific volume of water. Depending on the volume of the pipette, the absorbance of the solution will read a predetermined number. The pipette can then be adjusted, and the calibration repeated.

5. Centrifuges

- a. **Centrifuges** accelerate gravitational separation of substances differing in their masses. **Centrifugal force** is dependent upon several parameters, including mass and the speed and radius of rotation. Centrifuges are used to separate blood cells from serum or plasma, separate particulate matter in urine, and separate two liquid phases of different densities.
- b. Centrifuge speed should be checked periodically with a **tachometer** for accuracy.

c. Types of centrifuges

- 1) **Horizontal-head or swinging-bucket centrifuges** allow the tubes to attain a horizontal position in the centrifuge when spinning and a vertical position when the head is not moving.
- 2) **Fixed-angle or angle-head centrifuges** have angled compartments for the tubes and allow small particles to sediment more rapidly.
- 3) **Ultracentrifuges** are high-speed centrifuges used to separate layers of different specific gravities. They are commonly used to separate lipoproteins. The chamber is generally refrigerated to counter heat produced through friction.

6. **Balances and weighing**

- a. **Mass** is a physical property of matter. A balance compares the mass of an unknown against a known mass.
- b. NIST recognizes five types of calibration weights for assessing the accuracy of balances. **Class S weights** are used to check **analytical balances** for proper calibration. **Class M weights** have the quality of a **primary standard** and are used to check the accuracy of other weights.
- c. Types of balances
 - 1) **Double-pan balance** has a single beam with arms of equal length. Standard weights are added manually to the pan on the right side to counterbalance the weight of the object on the left-side pan.
 - 2) **Single-pan balance** has arms of unequal length. The object is placed on a pan attached to the shorter arm. A restoring force is applied mechanically to the other arm until the indicator is balanced.
 - 3) **Electronic balance** utilizes electromagnetic force to replace the weights as the counterbalance, with the force being proportional to the weight on the pan.

7. **Thermometers**

- a. There are three types of thermometers: **liquid-in-glass** (replaces mercury type), **digital**, and **electronic (thermistor probe)**.
- b. Thermometers should be **calibrated** using an NIST-certified thermometer. NIST provides an **SRM thermometer** with several calibration points, such as 30°C and 37°C.

C. **Sources and Control of Preanalytical Error**

1. **Preanalytical variation** includes:

- a. **Cyclic variation:** Changes in analyte concentration occur at different times during the day, week, or month
- b. **Diurnal variation:** Variation according to sleeping and waking times
- c. **Circadian variation:** Occurs during a 24-hour period
- d. **Circannual variation:** Occurs twice a year; related to seasonal changes in climate and diet (elevated in the summer, decreased in the winter)

e. **Physical variables**

- 1) **Exercise:** May cause alteration of serum potassium, phosphate, creatinine, and protein values
- 2) **Eating:** Causes increased serum glucose, triglycerides, etc.
- 3) **Stress:** May cause alteration of serum cortisol (increase), total cholesterol (increase), and even decrease hormone production of pituitary hormones and aldosterone

f. **Blood collection technique errors** in preservatives and/or anticoagulants, specimen type, or drawing technique

- 1) **Short draws** for coagulation studies are not acceptable.
- 2) **Proper anticoagulants**, plain red top tubes, or gel separator tubes must be selected based on the testing to be done.
- 3) **Stasis** caused by tourniquet use and repeated fist clenching, as well as improper drawing techniques, can lead to increased serum potassium, proteins, and metabolic by-products, as well as hemolysis of red blood cells.
- 4) **Hemolysis** causes false increase in serum levels of lactate dehydrogenase (LD), potassium, and magnesium, as well as a decrease in sodium.
- 5) **Lipemia** interferes with assays for a number of analytes.
- 6) **Drawing from a vein receiving intravenous (IV) fluid** dilutes blood analytes but increases the value of analytes present in the IV fluid (e.g., sodium, chloride, or glucose).

g. **Patient identification, sample identification, and chain of custody** are major concerns in specimen collection. Proper protocol must always be followed.

h. Although **sample transport** is always important, it is of special concern for accurate analysis of some analytes, such as plasma ammonia, plasma lactate, and blood gases/pH. Specimens for ammonia and lactate analysis should be placed on ice for transport to the laboratory. Blood gas/pH specimens drawn in plastic syringes should be transported immediately to the laboratory for analysis.

i. **Sample processing** involves logging the specimen into a laboratory information system (LIS) and assigning the sample an identification number, sorting and delivering specimens to various departments for testing, centrifuging to separate serum or plasma from red blood cells, and removing serum or plasma from red blood cells (if not in a gel separator tube).

j. **Sample storage**

- 1) **Separate serum or plasma from red blood cells** as soon as possible, and preferably within 2 hours of blood draw (may need to be sooner for some analytes). Gel separator tubes are commonly used in hospital situations, and they provide a good alternative for off-site collection provided a centrifuge is available (physician offices, clinics).

- 2) **Serum or plasma** can generally be **stored** at 2–8°C for 2–3 days; for long periods, storage at –20°C is recommended for many analytes.

D. Phlebotomy

1. Patient and collection preparation

- a. **Introduction:** Phlebotomy personnel should smile, introduce themselves, explain the procedure, be courteous, and act professionally.
- b. **Identify the patient.**
 - 1) Ask the patient/client to state his or her name.
 - 2) For an inpatient, compare the patient's name and identification number on the patient's identification band to the laboratory requisition. Electronic scanners may be used to scan patient identification band for confirmation.
 - 3) If the patient is an inpatient and an identification band is not present, the patient's nurse or physician must be asked to identify the patient. The name of the nurse or physician must be recorded on the laboratory requisition.
 - 4) If the patient is an outpatient without an identification band, the patient must provide two unique identifiers (e.g., name and date of birth) for proper identification.
- c. **Select the venipuncture site:** The preferred collection site includes the median cubital vein and the cephalic vein; as a last resort, the basilic vein can be used but caution must be taken because of its close proximity to the median nerve and the brachial artery.
- d. **Assemble all necessary equipment.**
 - 1) Gloves, alcohol swabs (betadine for blood cultures and alcohol levels), gauze, tape or band aids, evacuated blood collection tubes, needles, needle holders with safety, and tourniquets (Velcro or latex band; alternative nonlatex, **nitrile** materials should be **used** because of **latex allergies**). Lancets are used for finger sticks.
 - 2) When selecting blood collection tubes, use tubes with the smallest volume necessary for testing to prevent **iatrogenic anemia**.
 - 3) Apply the tourniquet and palpate using two fingers to select the most appropriate collection site. The phlebotomist should **never leave** the **tourniquet** on the patient **more than 1–2 minutes**. Patient results will be affected; blood may become more concentrated because of blood flow stasis. If the tourniquet is left on the arm for a prolonged time period, it could also increase the chance of pain and discomfort to the patient and the possible formation of a hematoma.
 - 4) Needles are usually 20 or 21 gauge. A **higher number** means a **smaller** needle diameter.
 - 5) Assemble the needle and needle holder with the evacuated tube, or needle and syringe for blood cultures or collection from fragile veins. Because of

small-diameter needles (23 gauge), some phlebotomists prefer using the butterfly (winged collection set) method for difficult draws. It is, however, more difficult to collect large quantities of blood using a butterfly needle.

- e. **Perform the venipuncture.**
 - 1) Needle should enter the site at a 15- to 30-degree angle, with the bevel of the needle facing up.
 - 2) Advance the evacuated tube onto the needle; change tubes carefully without moving the needle. If the collection tube has an anticoagulant, invert 2–3 times before progressing to the next tube.
 - 3) Once good blood flow is established or the last tube has been advanced onto the needle, the tourniquet should be removed.
- f. **After the blood collection**
 - 1) Remove the needle and then immediately apply pressure to the site using a gauze pad.
 - 2) Engage the safety on the needle immediately and discard needle into sharps container. **Phlebotomists should never recap contaminated needles.**
 - 3) **Invert tubes** containing anticoagulant several times.
 - 4) If bar coding is not used, label all tubes completely. Although labeling requirements vary among facilities, most require date and time of collection, name or initials of phlebotomist, and patient's name and identification number.
 - 5) Dispose of all contaminated materials appropriately.
 - 6) Thank the patient, remove gloves, and wash hands before leaving the patient's room.
- g. **Always maintain patient confidentiality.**
2. **Types of evacuated blood collection tubes**
 - a. **Red stopper tubes contain no additives.**
 - 1) Used when serum is required for a test
 - 2) May be used for routine chemistries, therapeutic drug levels, immunohematology, and serology
 - b. **Lavender stopper tubes contain ethylenediaminetetraacetate (K_3 EDTA), an anticoagulant.**
 - 1) EDTA ratio is 1.5 mg/1 mL of whole blood. Coagulation is prevented by removing ionized calcium (chelation), which forms an insoluble calcium salt.
 - 2) Used in hematology for complete blood count, slide preparation, and other routine hematology procedures; also used in immunohematology and for some special chemistry assays
 - 3) Alternative formulation of K_2 EDTA (pink stopper tubes) may be used in immunohematology.
 - c. **Blue tubes contain sodium citrate, an anticoagulant.**
 - 1) Sodium citrate (3.2%) in a 1:10 ratio, 1 part sodium citrate to 9 parts whole blood

- 2) Prevents coagulation by removing ionized calcium (chelation)
 - 3) Used for **coagulation studies** (e.g., prothrombin time and activated partial thromboplastin time)
 - d. **Green** tubes contain **heparin, an anticoagulant**.
 - 1) Several forms of heparin are available (e.g., lithium heparin and sodium heparin).
 - 2) Heparin ratio is 0.2 mL/1 mL of whole blood.
 - 3) Prevents coagulation by inactivating thrombin
 - 4) Used for routine and special chemistry and cytogenetic testing
 - e. **Speckled, tiger, or marbled** top **serum** separator tubes (SSTs) contain a **gel separator** and often contain **clot activators**.
 - 1) The **separator** is a **thixotropic gel** that forms a barrier between the cells and the serum during centrifugation.
 - 2) These tubes are useful when serum is needed; they are used frequently in clinical chemistry for a number of assays. Serum separator tubes are not recommended for immunohematology or drug levels.
 - f. **Speckled, tiger, or marbled** top **plasma** separator tubes (PSTs) contain a **gel separator** and an **anticoagulant**. In some cases, the PST stopper color is **green**, which indicates that **lithium heparin** is the anticoagulant.
 - 1) The **separator** is a **thixotropic gel** that forms a barrier between the cells and the plasma during centrifugation.
 - 2) These tubes are useful when plasma is needed. Plasma separator tubes reduce the time needed for clot formation and thus speed up the assay process. They are used frequently in clinical chemistry for a number of assays but are not recommended for immunohematology.
3. **Correct order of draw**
- a. To **prevent anticoagulant carryover** and contamination when using a needle and needle holder for blood collection, the following order of draw should be used: **sterile specimen** (blood culture), **light blue** top (sodium citrate; see below), **plain red** top or **SST, green** top (heparin or heparin PST), and **lavender** top (EDTA).
 - b. When using the butterfly (winged collection set) to collect a sodium citrate tube, if it is the first tube to be collected, then a discard tube (plain red or another sodium citrate tube) must be collected first to remove the air from the tubing. Sodium citrate tubes must be completely filled to ensure the correct plasma:anticoagulant ratio, or the test results could be compromised.

II. LABORATORY QUALITY ASSESSMENT

A. Definitions

1. **Total quality management (TQM)** is a managerial process that focuses on improvement of the quality of all factors that affect laboratory testing and performance. It consists of five elements: quality laboratory process, quality control, quality assessment, quality improvement, and quality planning.

2. **Quality assessment (QA):** This is a systemic laboratory program, encompassing preanalytical, analytical, and postanalytical factors, that monitors excessive variation in specimen acceptability, test methodologies, instruments, reagents, quality control, and personnel competencies. This process is used to ensure accurate patient test results.
3. **Preanalytical error** occurs during sample collection and transport before sample analysis and can include sample preparation and storage conditions.
4. **Analytical error** occurs during the testing process and includes problems related to reagents, instruments, controls, calibration, performance of personnel, etc.
5. **Postanalytical error** occurs after the test is performed and refers to clerical errors, reporting of results, test interpretation, etc.
6. **Accuracy** is a measure of a laboratory test result's closeness to the true value.
7. **Precision** is realized when repeated laboratory test results yield the same number; reproducibility.
8. **Reliability** refers to the ability of laboratory testing to maintain accuracy and precision over an extended period of time.
9. **Quality control (QC):** A system used to monitor the analytical process to detect and prevent errors that would impact on the accuracy and precision of laboratory test results; includes both statistical and nonstatistical parameters.
 - a. **Internal QC** is performed by laboratory personnel using control materials of known values and comparing the control values to established, acceptable ranges. The control material values are assessed using Levey-Jennings control charts and Westgard multirules to detect errors.
 - b. **External QC** is performed by laboratory personnel when analyzing specimens sent to the laboratory by an external agency, and the results generated are submitted to the agency for assessment. This type of assessment is known as **proficiency testing**. It is required by federal regulations for all laboratories providing results for human diagnosis and/or treatment.
10. **Linearity check** determines the lowest and highest values that can be accurately measured by a particular method. This is an example of a nonstatistical QC parameter.
11. **Random errors** affect precision, are unable to predict because they have no known pattern, and may alternate between a positive or negative direction.
12. **Systematic errors** are predictable and cause a constant difference in results that are consistently positive or negative or stay the same. Such errors may be due to incorrect calibration, deteriorated reagents, instrument malfunction, etc.
13. **Delta check** assesses the patient's most recent result for a particular test as compared to the patient's previous value; the difference between the test results (delta) is calculated and compared to established limits.
14. **Reference ranges** are determined by each laboratory to fit their particular population. Intervals are generally constructed by adding and subtracting 2 standard deviations from the mean.

15. **Standard** is material of known concentration (should be traceable to NIST) that is used to calibrate an instrument or develop a standard curve.
16. **Control** is material of known value that is analyzed with patient samples to determine acceptability of results.
 - a. **Assayed control:** Values are assigned by the manufacturer.
 - b. **Unassayed control:** Values are determined by each individual laboratory for their methods/instruments.
17. **Westgard multirules** are statistical “rules” applied to graphical summaries of numerical quality control data to assess the acceptability of such data.
18. **Six Sigma** is a data-driven, business approach to performance improvement; it is oriented toward process identification and process improvement.
19. **Lean principles** are an improvement trend to make work faster by providing ways to streamline through the removal of waste.
20. **ISO 9000 Standards** were established by the International Organization for Standardization as a series of four standards for quality management.

B. Specimen Quality

1. Test result quality depends on the quality of the sample submitted.
2. Specimen quality depends on:
 - a. Patient preparation
 - b. Labeling procedures
 - c. Timing of specimen collection
 - d. Special collection instructions
 - e. Specimen handling and transport requirements
 - f. Criteria for unacceptable specimens

C. Operating Instructions

1. **Laboratory procedures** should contain the following information: test name, method principle, significance of test, patient preparation, test specimen requirements, equipment and materials needed, reagent preparation, test procedure, calculations, quality control procedures, reference intervals, panic values, limitations of the procedure, and references, including the instrument user’s manual. Each procedure must be reviewed, signed, and dated annually.
2. Instrument user’s manual and instrument maintenance manual must be available, and all maintenance performed must be documented.

D. Selecting Instruments

1. Selection criteria should include instrument cost, reagent cost, throughput, technical support, personnel training, method linearity, range of methods available, test methodologies, analytical sensitivity and specificity, etc.
2. Instruments are evaluated to determine instrument and method accuracy, precision, systematic error, linearity, and calibration stability.

E. Statistical Analysis

1. **Arithmetic mean** (\bar{x}) of a set of numbers is obtained by adding all the numbers in the set and dividing the sum by the number of values in that set.
2. **Median** is the middle value in a set of numbers that are arranged according to their magnitude.
3. **Mode** is the most frequently obtained value in a set of numbers.
4. **Standard deviation** (s) reflects the variation of data values around the mean.
5. **Variance** (s^2) reflects dispersion around the mean and is the square of the standard deviation.
6. **Coefficient of variation (CV)** reflects random variation of analytical methods in units that are independent of methodology, because it is a percentage comparison of the standard deviation divided by the mean.
7. **Normal distribution** is a symmetric distribution about the mean, where 95.45% of the values lie within $\pm 2s$ and approximately 5% will normally fall outside.
8. The **sensitivity** of a test is the percentage of individuals with a specific disease that are correctly identified or predicted by the test as having the disease.
9. The **specificity** of a test is the percentage of individuals without the specific disease that are correctly identified or predicted by the test as not having the disease.
10. **Predictive value** of a test utilizes the parameters of test sensitivity and specificity as well as disease prevalence (i.e., incidence of a disease or condition).
 - a. **Positive predictive value** is the percentage of people with positive test results who have the disease.
 - b. **Negative predictive value** is the percentage of people with negative test results who do not have the disease.

F. Reference Intervals

1. **Reference intervals (ranges)** are calculated for each laboratory's menu of tests. Each laboratory serves a unique population, so the reference intervals must be determined for that population.
2. Use a **minimum of 20 specimens** from "healthy" people to determine analyte values, calculate the mean and standard deviation, and compare to the reference interval suggested by the manufacturer.
3. **Preferably**, analyte values should be determined using a **minimum of 120 specimens** from healthy people in each relevant sex and age category.
4. Calculate the **mean** and **standard deviation**.
5. **Reference intervals** are calculated using the **mean + 2 standard deviations** (high value) and the **mean - 2 standard deviations** (low value) to include 95% of the "healthy" population.

G. Internal Quality Control

1. **Purpose:** It is a comprehensive program involving statistical analysis of control materials, which are analyzed with a batch of patient samples to determine acceptability of the run.
2. **Control material**
 - a. Commercially manufactured **lyophilized or liquid materials** that have the **same matrix** as patient specimens and are used to evaluate the test process.
 - b. Control materials are handled exactly like patient specimens: Analysis conditions (incubation time, analysis temperature, calculation, etc.) and preanalysis conditions if warranted (precipitation, protein-free filtrate, etc.).
 - c. Control materials are selected so that values will be at **medically significant** levels. Generally, one control will have a value within the reference interval, and a second control will have an abnormal (elevated) value.
 - d. It is preferred that the **same lot number** of control material be purchased and used for at least a 1-year period.
 - e. Lyophilized control material must be **accurately reconstituted** according to the manufacturer's directions to avoid vial-to-vial variability. The stability of the reconstituted material is important.
 - f. For **qualitative controls**, use materials that will provide **both negative and positive results**.
3. **Data evaluation procedures for control materials**
 - a. **Levey-Jennings control chart** is constructed monthly for each control material using the mean ± 3 standard deviations to construct a graph that allows visual detection of shifts and trends. The control value is plotted versus the established range, with the **acceptable control range represented by ± 2 standard deviations**.
 - 1) Control values that exceed the mean ± 2 standard deviations are generally considered unacceptable and alert personnel to investigate the cause.
 - 2) **Trend** is a gradual change in the mean that is reflected as either a decrease or increase of consecutive control values (generally the number of consecutive observations signifying a trend is six or more). The change occurs only in one direction.
 - 3) **Shift** is a sudden change in the mean that is reflected as consecutive control values above or below the mean.
 - 4) **A loss of precision** is obvious on the chart when control values become more dispersed.
 - b. **Westgard multirule** is a control procedure that utilizes control rules to assess numerical quality control data; the control rules establish the limits for data rejection in a system with two controls. Other rules apply when three controls are used.
 - 1) **1_{2s}**—1 control value exceeds the mean ± 2 standard deviations; warning rule that triggers inspection of control values using the other rejection

rules that follow; only rule that is not used to reject a run; results are reportable

- 2) 1_{3s} —1 control value exceeds the mean ± 3 standard deviations; detects random error
 - 3) 2_{2s} —2 consecutive control values exceed the same 2 standard deviation (s) limit (same mean $+2 s$ or same mean $-2 s$); detects systematic error
 - 4) R_{4s} —1 control value in a group exceeds the mean $+2 s$ and a second control value exceeds the mean $-2 s$, creating a 4 standard deviation spread; detects random error
 - 5) 4_{1s} —4 consecutive control values are recorded on one side of the mean and exceed either the same mean $+1 s$ or the same mean $-1 s$; detects systematic error
 - 6) 10_x —10 consecutive control values are recorded on one side of the mean (either above or below the mean); detects systematic error
4. **Youden plot** is a graphical technique for analyzing interlaboratory data when each laboratory has made two runs on the same analyte or one run on two different analytes. The plot identifies **within-laboratory** and **between-laboratory** variability.

H. External Quality Control

1. **External quality control** refers to a program where a clinical laboratory contracts with an agency (e.g., College of American Pathologists or American Association of Bioanalysts) to receive and assay samples, the concentration of which is unknown to the participating clinical laboratory. The same samples are sent by the agency to reference laboratories for analysis for the purpose of establishing target values and ranges of acceptability. The results generated by the participating clinical laboratory are sent to the agency for comparison to the values established by the reference laboratories for the purpose of assessing the clinical laboratory's level of performance. This is known as proficiency testing.
2. **Proficiency testing:** An agency sends proficiency samples to a clinical laboratory to analyze, and the results generated are assessed by the agency for accuracy to determine the performance of the laboratory. Assessment reports are sent to participating laboratories to assist with performance analysis and test method and equipment selection. Federal CLIA '88 (Clinical Laboratory Improvement Amendments '88) requires that all laboratories performing human testing for diagnosis and/or treatment must use proficiency testing for all analytes it reports. Failure to comply can result in sanctions, including a complete closure of the laboratory.
3. **Proficiency samples** have a similar matrix to patient specimens, are generally shipped in a lyophilized form with diluent, and are utilized in proficiency testing programs.

4. **Limitations of external quality control programs**

- a. Some laboratories will **treat proficiency samples differently** than normal patient specimens (i.e., special handling, running controls before and after each proficiency sample, calibrating the assay before running the proficiency sample, special selection of personnel to perform the assay, etc.). Such deviation from routine workload procedures will not reflect the accuracy and precision of the laboratory.
 - b. Proficiency samples **do not reflect the preanalytical component** of patient identification, collection, and handling procedures. There could be problems in these areas that an external quality control program is not designed to address.
5. For a clinical laboratory to comply with **CLIA '88**, the laboratory must successfully participate in proficiency testing. In turn, the agencies that provide proficiency testing to clinical laboratories must be approved by the Centers for Medicare and Medicaid Services (CMS).

III. LABORATORY SAFETY

A. Regulatory Oversight

1. **Occupational Safety and Health Administration (OSHA)**
 - a. Federal agency charged with the enforcement of safety and health legislation
 - b. **Occupational Safety and Health Act of 1970** makes employers responsible for providing a safe and healthy workplace for their employees.
 - c. **Hazardous Communication Programs**, also known as the **Right to Know Standard**: The purpose of this standard is to ensure that chemical hazards in the workplace are identified and information concerning these hazards is communicated to employers and employees.
2. **Centers for Disease Control and Prevention (CDC)**: Federal agency that publishes numerous safety standards
3. **The Joint Commission**: Issues standards and grants accreditation to improve the safety and quality of care provided to the public through inspections of healthcare facilities
4. **College of American Pathologists (CAP)**: Issues standards and offers accreditation through inspections

B. Safety Program

1. Accreditation organizations require clinical laboratories to have a formal safety program. The program needs to ensure that the laboratory environment meets approved safety standards.
2. **Safety officer or chair of the safety committee**: Responsibility is to implement and maintain a safety program

3. **Chemical hygiene officer (CHO):** OSHA requires that laboratories have a designated CHO whose responsibility is to provide technical guidance in the development and implementation of the chemical hygiene plan.
4. **Material safety data sheet (MSDS)** defines the toxicity of a chemical. The MSDS must be provided by the manufacturer and includes the following information:
 - a. Physical data such as boiling point, vapor pressure, and specific gravity
 - b. **Threshold limit value (TLV):** Exposure allowable for an employee during one 8-hour day
 - c. Spill, disposal, and first aid procedures
 - d. Personal protective equipment required to handle the chemical
 - e. Additional toxicity must be listed; this would include identifying chemicals as a **carcinogen** (substance or agent causing cancer), a **mutagen** (causes changes in DNA), or a **teratogen** (causes birth defects).
5. **Safety inspections**
 - a. The laboratory should have a safety committee or inspection team periodically inspect the laboratory.
 - b. Several federal, state, and private accreditation organizations (e.g., CAP and The Joint Commission) conduct inspections of healthcare facilities. These inspections may be regularly scheduled or unannounced. Inspections may also follow a complaint filed against a facility.

C. Personal Safety

1. **Wash hands** before leaving the laboratory and after taking off gloves.
2. **Do not mouth pipet.**
3. Tie back long hair and avoid loose sleeves/cuffs, rings, bracelets, etc.
4. **Do not apply cosmetics** in the laboratory.
5. **Eating and drinking are forbidden in the laboratory.**
6. **Housekeeping**
 - a. Maintain orderly work areas
 - b. Keep aisle-ways clear and free of tripping hazards
 - c. Keep floors dry to avoid slipping; attend to spills immediately

D. Personal Protective Equipment

1. OSHA requires that employers provide all necessary **personal protective equipment** (PPE) to employees.
2. **Eye protection:** Goggles and face shield
3. **Protective clothing**
 - a. The **laboratory coat** is designed to protect the clothing and skin from chemicals that may be spilled or splashed. It should be worn buttoned up and with the sleeves extended to the wearer's wrist.
 - b. **Foot protection** is designed to prevent injury from corrosive chemicals or heavy objects. If a corrosive chemical or heavy object were to fall on the

floor, the most vulnerable portion of the body would be the feet. For this reason, shoes **that completely cover and protect** the foot are worn in the laboratory.

4. **Hand protection:** Heat-resistant gloves for handling hot or cold objects (e.g., dry ice) and latex or nitrile gloves to prevent exposure to biological hazards must be available. Selection of protective gloves is based on chemical hazard and the tasks involved.

E. Safety Equipment

1. Individual storage containers

- a. Selecting the best means of storage for chemical reagents will, to a great extent, depend on that reagent's compatibility with the container. A safety can is an approved container of no more than 5-gallon capacity. It has a spring-closing lid and spout cover and is designed to safely relieve pressure buildup within the container.
- b. **Sharps containers:** Hard containers for the disposal of sharp objects such as used phlebotomy needles, broken contaminated glass, and pipettes

2. **Eye wash stations** must be inspected and tested periodically for proper function.

3. **Safety showers** provide an effective means of treatment in the event that chemicals are spilled or splashed onto the skin or clothing. They must be inspected and tested periodically for proper function.

4. Refrigerators

- a. Standard refrigeration units are **not appropriate for storing flammable materials**.
- b. **Laboratory refrigerators are not appropriate for storing food for consumption.** Each refrigerator and freezer must be labeled, "No food or beverages may be stored in this refrigerator."
- c. Each refrigerator and freezer must be monitored daily to ensure proper functioning.

5. **Alarms** are designed so that endangered personnel are alerted. All individuals should become familiar with the exact location of the fire alarm stations nearest to their laboratory.

6. Chemical spill kits

- a. Laboratories are equipped with clean-up kits for various types of spills. Wear the appropriate PPE (i.e., gloves, goggles) when cleaning up spills.
- b. **Acid spills**
 - 1) Apply neutralizer (or sodium bicarbonate) to perimeter of spill.
 - 2) Mix thoroughly until fizzing and evolution of gas ceases.
 - 3) Transfer the mixture to a plastic bag, tie shut, fill out a waste label, and place in a fume hood.
- c. **Solvent spills**
 - 1) Apply activated charcoal to the perimeter of the spill.

- 2) Mix thoroughly until material is dry and no evidence of solvent remains.
- 3) Transfer absorbed solvent to a plastic bag, tie shut, and place in fume hood.

7. **Chemical fume hood**

- a. The only safe place to work with some highly toxic and volatile chemicals
- b. Partially enclosed ventilated work space for volatile chemicals
- c. Chemical fume hoods are generally ducted and vent air outside the building.
- d. Fume hoods are not to be used for the storage of hazardous chemicals.

8. **Biological safety cabinets**

- a. **Class 1 cabinets** have an open front and are under negative pressure. Air is exhausted into the room after passing through high-efficiency particulate air (HEPA) filters.
- b. **Class 2 cabinets** provide added protection by forcing HEPA-filtered air downward at the front of the cabinet where the laboratorian is working. The air can be exhausted into the room (**Class 2A**) or outside the building (**Class 2B**).
- c. **Class 3 cabinets** are gas-tight. The interior of the cabinet is only accessible through glove ports.
- d. Chemical fume hoods and biological safety cabinets **cannot** be used interchangeably. Fume hoods will not protect workers from infectious agents, and biological safety cabinets may not protect against chemical vapors. In addition, chemicals can damage the HEPA filters in biological safety cabinets.

F. Waste Collection and Disposal

1. Discard all nonsharp biohazardous substances into biohazard bags.
2. Dispose of used tubes in biohazard bags.
3. Dispose of swab wrappings, band aid wrappings, used paper towels, kit boxes, and any other nonbiohazardous waste into regular trash bags.
4. Do not discard nonbiohazardous waste into red biohazard bags.
5. Store chemicals in appropriate chemical can. Chemicals should not be poured down sink drains.

G. Mandated Plans

1. **Chemical hygiene plan**

- a. OSHA requires laboratories to have a chemical hygiene plan.
- b. List of responsibilities of employers and employees
- c. Chemical inventory list
- d. Copies of the MSDSs must be readily available.

2. **Exposure control plan**

- a. OSHA requires that all laboratories have an exposure control plan to minimize risk of exposure to **bloodborne pathogens** (BBPs).
- b. Regulates disposal of medical waste

3. **Ergonomic plan**

- a. CAP requires laboratories to have an ergonomic plan to minimize risk of work-related musculoskeletal disorders.
- b. Avoid awkward posture, repetitive motion, and repeated use of force.
- c. Employer must provide training and appropriate equipment, and an assessment and documentation system.

4. **Transportation and shipping of clinical specimens**

- a. Laboratories are responsible for preventing people from being exposed to infectious agents during transport.
- b. The Department of Transportation (DOT), International Air Transport Association (IATA), and the International Civil Aviation Organization (ICAO) developed strict guidelines for the handling and shipping of hazardous materials. Only special approved shipping containers can be used. Only individuals who have received training and have a permit are allowed to ship hazardous material.

H. **Laboratory Hazards**

1. **The United Nations (UN)** established nine classes of hazardous materials.

- a. Class 1—explosives
- b. Class 2—compressed gases
- c. Class 3—flammable liquids
- d. Class 4—flammable solids
- e. Class 5—oxidizer materials
- f. Class 6—toxic materials
- g. Class 7—radioactive materials
- h. Class 8—corrosive materials
- i. Class 9—miscellaneous materials not classified elsewhere

2. **Warning labels**

- a. The DOT requires all chemicals shipped in the U.S. have labels based on the UN hazardous material classification.
- b. DOT labels are diamond shaped with the classification number in the bottom corner. The hazard is also identified in words along the horizontal axis of the label.
- c. The DOT label is only on the shipping container. Once received, the laboratory must label each individual container in the shipping container.
- d. Although OSHA mandates the use of labels or appropriate warnings, no single uniform labeling system exists for hazardous materials.
- e. The **National Fire Protection Association (NFPA)** developed the **704-M Identification System**, which most laboratories use.
 - 1) The labels are diamond shaped, and each quadrant has a different color: **blue**—health; **red**—flammability; **yellow**—reactivity; and **white**—special information. The chemical is classified 0–4 (least

hazardous to most hazardous) in the areas of health, flammability, and reactivity.

- 2) The chemical can be identified as a poison, water reactive, etc. in the white quadrant.

3. Chemical hazards

- a. Approved spill kits must be nearby.
- b. Concentrated acids must be diluted by adding them to water in the sink.
- c. Label all containers **before** adding the chemical.
- d. Some chemicals can become more hazardous if stored for a prolonged time. **Picric acid** has the potential to form peroxides if stored for a long period of time and not used. The material can become **shock sensitive**, with the potential to explode if bumped.
- e. **Sodium azide**, a carcinogen, is sometimes used as a preservative in laboratory reagents. When disposed of in the sewer, the accumulation of copper and iron salts of azide may occur. These metallic salts are explosive, especially when subjected to mechanical shock.
- f. Working with **carcinogens** requires special precautions such as using a fume hood, wearing rubber gloves and a respirator, and cleaning contaminated glassware with a strong acid or organic solvent.
- g. **Chemical containers** made of glass should be transported in rubber or plastic holders that will protect them from breakage.

4. Fire hazards

- a. **Flammability** is a measure of how easily a gas, liquid, or solid will ignite and how quickly the flame, once started, will spread. **Flammable** and **inflammable** both mean “to catch fire easily.”
- b. **Flammable liquids themselves are not flammable; rather, the vapors from the liquids are combustible.** There are two physical properties of a material that indicate its flammability: flash point and volatility (boiling point).
 - 1) The **flash point** of a material is the temperature at which a liquid (or volatile solid) gives off vapor in quantities significant enough to form an ignitable mixture with air.
 - 2) The **volatility** of a material is an indication of how easily the liquid or solid will pass into the vapor stage. Volatility is measured by the boiling point of the material—the temperature at which the vapor pressure of the material is equal to the atmospheric pressure. Volatile solvents should be stored in small amounts in an explosion-proof refrigerator.
 - 3) The flash point of **flammables** is designated as less than 100°F, and that of **combustibles** as greater than 100°F.
- c. Xylene, ethanol, methanol, and acetone are flammable chemicals commonly used in clinical laboratories that must be stored in a **flammable liquid safety cabinet**.
- d. Some materials are **pyrophoric**, meaning that they can ignite spontaneously with no external source of ignition. Potassium metal, for example, can react with the moisture in air.

e. **Storage**

- 1) **Flammable materials should never be stored near acids.**
- 2) **Storage areas should be cool** enough to prevent ignition in the event that vapors mix with air. Adequate ventilation should be provided to prevent vapor buildup.
- 3) **Avoid storage of flammable materials in conventional** (non-explosion-proof) **refrigerators.** Sparks generated by internal lights or thermostats may ignite flammable material inside the refrigerator, causing an extremely dangerous explosion hazard.
- 4) Be aware of **ignition sources** in your laboratory area (heat sources, electrical equipment).

f. **Handling**

- 1) Use gloves and safety goggles when handling flammable liquids or vapors.
- 2) Dispensing of flammable or combustible liquids should only be done in a fume hood or in an approved storage room.
- 3) Do not use water to clean up flammable liquid spills.

g. **Extinguishers**

- 1) Extinguishers are classified according to a particular fire type and are given the same letter and symbol classification as that of the fire.
 - a) **Type A**—combustibles: wood, cloth, paper, rubber, and plastics
 - b) **Type B**—flammable liquids: oil, grease, and paint thinners
 - c) **Type C**—energized electrical equipment: electrophoresis
 - d) **Type D**—combustible metals: magnesium, titanium, sodium, lithium, potassium
- 2) **Multipurpose extinguishers** are highly recommended because they are effective against Type A, B, and C fires.

5. **Biological hazards**

- a. The National Institutes of Health guidelines describe four levels of biosafety depending upon the biological agents isolated or studied. The levels are based on the virulence of the agents and the availability of effective treatments and vaccines.
 - 1) **Biosafety Level 1** laboratories handle agents that have no known potential for infecting healthy people.
 - 2) **Biosafety Level 2** laboratories are those laboratories that work with microorganisms associated with human diseases that are rarely serious and for which preventive or therapeutic interventions are often available. Most clinical microbiology laboratories are Level 2.
 - 3) **Biosafety Level 3** is recommended for materials that may contain viruses not normally encountered in a clinical laboratory and for the cultivation of mycobacteria. Clinical laboratories offering these services must have a Level 3 facility. Working with mycobacteria requires the use of **N95 HEPA filter respirators**; surgical masks are not acceptable.

- 4) **Biosafety Level 4** is required for work with dangerous and exotic agents that pose a high risk of aerosol-transmitted laboratory infections and life-threatening disease for which effective treatments are limited.
 - b. **Exposure risks**
 - 1) Accidental punctures with needles
 - 2) Spraying (aerosols) or spilling infectious materials onto desktop or floor
 - 3) Cuts or scratches from contaminated object
 - 4) Centrifuge accidents: Aerosols, broken tubes, etc.
 - c. **Bloodborne pathogens** are transmitted through contact with infected blood and body fluids and include **human immunodeficiency virus**, **hepatitis B virus** (HBV), and **hepatitis C virus**.
 - d. Use **standard precautions** and treat all blood or potentially infectious body fluids as if they are contaminated. Avoid contact whenever possible, and whenever it's not, wear personal protective equipment.
 - e. All surfaces, tools, equipment, and other objects that come in contact with blood or potentially infectious materials **must be decontaminated** and sterilized as soon as possible. Decontamination is recommended with **5.25% chlorine bleach** (sodium hypochlorite; NaOCl) solution, a 1:10 dilution of household bleach. The diluted bleach solution should be made daily.
 - f. OSHA requires that employers offer employees **HBV vaccine** if their regular duties present a potential for exposure to the virus.
 - g. Before leaving the laboratory, laboratorians should wipe the countertop with a disinfectant, wash their hands in an antiseptic soap, and remove their laboratory coat.
 - h. Microbiology laboratories are engineered to maintain negative air pressure with respect to the administrative areas. This maintains airflow into the laboratory, minimizing the risk of airborne pathogens exiting the laboratory when a door is opened.
6. **Compressed gases**
- a. Transportation of compressed gases is regulated by the DOT.
 - b. NFPA labels should be attached to each cylinder.
 - c. Gas cylinders should be secured onto a hand truck for transporting.
 - d. Gas cylinders must be stored in a vertical position chained to a wall.
 - e. When in use, gas cylinders must be securely fastened to a wall or laboratory bench.



review questions

INSTRUCTIONS Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

General Laboratory Principles

- Which of the following is an alumina-silicate glass that is at least six times stronger than borosilicate and is resistant to alkaline etching and scratching?
 - Kimax
 - Pyrex
 - Corning boron free
 - Corex
- The National Institute of Standards and Technology (NIST) requires that volumetric pipettes and flasks be certified as
 - Class A
 - Class B
 - Class C
 - Class D
- Which of the following desiccants is the most hygroscopic?
 - Silica gel
 - Alumina
 - Barium oxide
 - Magnesium perchlorate
- Lavender top blood collection tubes are used for complete blood counts. What is the anticoagulant in these tubes?
 - EDTA
 - Heparin
 - Sodium citrate
 - Sodium oxalate
- SI units are the designated units employed by the International System of Units. The unit class that encompasses the seven fundamental quantities of measurement is
 - Base
 - Primary
 - Derived
 - Elemental
- Which of the following parameters does *not* have an effect on centrifugal force?
 - Temperature of the centrifuge
 - Mass of the material being centrifuged
 - Speed of rotation
 - Radius of the centrifuge

7. What class of weights should be used to check the calibration of analytical balances?
 - A. J
 - B. M
 - C. P
 - D. S
 8. “To deliver” (TD) pipettes are identified by
 - A. Two etched bands near the top
 - B. Self-draining capacity
 - C. Dual-purpose pipette labels
 - D. Blue graduation levels
 9. If a laboratory needs to keep certain chemical materials dry, the apparatus used will be a
 - A. Buret
 - B. Desiccator
 - C. Separatory funnel
 - D. Vacuum
 10. Which of the following is an advantage of the angle-head centrifuge over the horizontal-head centrifuge?
 - A. Less air friction
 - B. Smaller increase in sample temperature during centrifugation
 - C. Can be operated at a higher speed
 - D. All the above
 11. The type of water desired for use in test methods requiring maximum accuracy and precision is
 - A. Distilled
 - B. Pure grade
 - C. Reagent grade
 - D. Special reagent water
 12. The speed of a centrifuge should be checked at least once every 3 months with a(n)
 - A. Tachometer
 - B. Wiper
 - C. Potentiometer
 - D. Ergometer
 13. The type of balance that uses an electro-magnetic force to counterbalance the load placed on the pan is a(n)
 - A. Trip balance
 - B. Class A balance
 - C. Class S balance
 - D. Electronic balance
 14. Which of the following blood collection tube tops would contain a serum separator gel?
 - A. Blue
 - B. Grey
 - C. Lavender
 - D. Speckled
 15. When performing a venipuncture, which of the following is the proper order of draw for evacuated blood collection tubes?
 - A. Sterile specimen, light blue top, and plain red top
 - B. Light blue top, plain red top, and sterile specimen
 - C. Lavender top, light blue top, plain red top
 - D. Green top, sterile specimen, and plain red top
- ### Laboratory Quality Assessment
16. What term applies to the sum of all the values in a set of numbers divided by the number of values in that set?
 - A. Median
 - B. Mode
 - C. Arithmetic mean
 - D. Geometric mean
 17. Calculate the coefficient of variation (percent) for a set of data where the mean (\bar{x}) = 89 mg/dL and 2 standard deviations (s) = 14 mg/dL.
 - A. 7.8
 - B. 7.9
 - C. 15.7
 - D. 15.8

18. What does the preparation of a Levey-Jennings quality control chart for any single constituent of serum require?
 - A. Analysis of control serum over a period of 20 consecutive days
 - B. 20 to 30 analyses of the control serum, on 1 day, in one batch
 - C. Analyses consistently performed by one person
 - D. Weekly analyses of the control serum for 1 month
19. A batch of test results is out of control. What should you do first?
 - A. Report the results to the physician first, and then look for the trouble.
 - B. Follow the “out-of-control” procedure specified for the test method.
 - C. Repeat the tests with a new lot of standards (calibrators).
 - D. Repeat the tests with a new lot of reagents.
20. In addition to utilizing Levey-Jennings charts, what other criteria should be applied to interpret internal quality control data?
 - A. Westgard multirule
 - B. Cusum
 - C. Linear regression
 - D. Youden
21. A new standard (calibrator) has been prepared in error at a lower concentration than that required for the test. How would such an error appear on a quality control chart?
 - A. Upward trend
 - B. Downward trend
 - C. Upward shift
 - D. Downward shift
22. The ± 2 standard deviation ($\pm 2 s$) range of acceptable values for a digoxin control is established as 2.0–2.6 ng/mL. On the average, the expectation that a value will be greater than 2.6 ng/mL is 1 in
 - A. 10
 - B. 20
 - C. 40
 - D. 100
23. What is the purpose of a Youden plot?
 - A. Compares results on two control specimens, low and high controls, for the same analyte analyzed by several laboratories
 - B. Evaluates the validity of daily results on a single control specimen over a period of 30 days
 - C. Compares results on a single control specimen by two different methods for the same analyte
 - D. Evaluates the validity of daily results of two control specimens within a single laboratory
24. If the therapeutic range for the gentamicin assay is a trough level of less than 2 $\mu\text{g/mL}$ and a peak level of 5–8 $\mu\text{g/mL}$, what would be appropriate mean values for two control levels (in micrograms per milliliter) used to monitor the system?
 - A. 1 and 2
 - B. 1 and 3
 - C. 1.5 and 6
 - D. 5 and 6
25. Which of the following monitoring factors would *not* be included in a laboratory’s quality assessment program?
 - A. Scheduling of staff
 - B. Specimen collection and identification
 - C. Accuracy and precision of analyses
 - D. Preventive maintenance of instruments

26. On a quality control chart, when would a statistical out-of-control situation requiring corrective action be suspected?
 - A. Six successive plots fall above and below the mean within $\pm 1 s$.
 - B. Six successive plots fall above and below the mean within $\pm 2 s$.
 - C. One plot falls within the area of $\pm 2 s$ to $3 s$ within a 20-consecutive-day span.
 - D. One plot falls outside the area of $\pm 3 s$ within a 20-consecutive-day span.
27. Which of the following would result in a sudden shift in daily values on a quality control chart?
 - A. Recalibrating the instrument when changing reagent lot numbers during an analytical run
 - B. Replacing the instrument's sample aspiration probe
 - C. Changing the spectrophotometer lamp in the middle of a sample run
 - D. Changing personnel operating the instrument
28. Which of the following terms refers to the measure of scatter of experimental data around the mean of a Gaussian (normal) distribution curve?
 - A. Median
 - B. Mode
 - C. Coefficient of variation
 - D. Standard deviation
29. The percentage of individuals without a specific disease who are correctly identified or predicted by the test as not having the disease describes
 - A. Sensitivity
 - B. Specificity
 - C. Positive predictive value
 - D. Negative predictive value
30. Which of the following terms refers to deviation from the true value caused by indeterminate errors inherent in every laboratory measurement?
 - A. Random error
 - B. Standard error of the mean
 - C. Parametric analysis
 - D. Nonparametric analysis
31. What is the following formula used to calculate? $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$
 - A. Coefficient of variation
 - B. Variance
 - C. Confidence limits
 - D. Standard deviation
32. Which of the following terms refers to the closeness with which the measured value agrees with the true value?
 - A. Random error
 - B. Precision
 - C. Accuracy
 - D. Variance
33. What percentage of values will fall between $\pm 2 s$ in a Gaussian (normal) distribution?
 - A. 34.13%
 - B. 68.26%
 - C. 95.45%
 - D. 99.74%
34. Which of the following terms refers to a measure of dispersion or spread of values around a central value?
 - A. Range
 - B. Validity
 - C. Variance
 - D. Coefficient of variation

35. Which of the following describes the ability of an analytical method to maintain both accuracy and precision over an extended period of time?
- Reliability
 - Validity
 - Probability
 - Sensitivity
36. What is the following formula used to calculate? $\frac{\sum(x - \bar{x})^2}{n - 1}$
- Coefficient of variation
 - Variance
 - Confidence limits
 - Standard deviation
37. Which of the following does *not* pertain to the characteristics and use of assayed control material?
- Has physical and chemical properties resembling test specimen
 - Contains preanalyzed concentrations of analytes being measured
 - Can be interchanged in terms of use with primary standards or calibrators
 - Concentrations of analytes should be in normal and abnormal ranges.
38. A group of physicians consistently complains that they are not receiving stat patient results quickly enough. The supervisor is likely to refer to which quality assessment variable?
- Specimen separation and aliquoting
 - Test utilization
 - Analytical methodology
 - Turnaround time
39. To provide independent validation of internal quality control programs, external surveys have been developed. Which of the following is a representative survey program?
- CLSI (Clinical and Laboratory Standards Institute)
 - ASCLS (American Society for Clinical Laboratory Science)
 - ASCP (American Society for Clinical Pathology)
 - CAP (College of American Pathologists)
40. A tech is scheduled to perform a specialized test that she/he is familiar with, but is not *exactly* certain of the steps required. What is the best course of action to take?
- Ask another tech to perform the test.
 - Consult the procedure manual and notify the supervisor.
 - Run the test as best as possible, being careful to note control values.
 - Reject the specimen.
41. A tech has completed the first run of morning specimens. She/he notices that the one control being used is outside $\pm 3 s$. What course of action should be taken?
- Release the results.
 - Repeat the control only, and if it comes in, release results.
 - Check equipment and reagents to determine source of error; repeat the entire analysis, including the control and patients; if the control value is within $\pm 2 s$, release results.
 - Repeat the control; if the same thing happens, attribute the cause to random error; release results.

42. Which of the following describes the Westgard multirule 2_{2s} ?
- A. Two control data points are within $\pm 2 s$.
 - B. One control data point falls outside $+2 s$ and a second point falls outside $-2 s$.
 - C. Two consecutive data points fall outside $+2 s$ or fall outside $-2 s$.
 - D. Two consecutive data points fall outside $+2 s$.
43. Which Westgard multirule applies to a situation where one control point exceeds the mean by $+2 s$ and a second control point exceeds the mean by $-2 s$?
- A. 1_{2s}
 - B. 2_{2s}
 - C. 4_{1s}
 - D. R_{4s}
44. Upon admission to the hospital, a chemistry profile is performed on a patient. The patient has a total bilirubin of 2.0 mg/dL. The next day a second chemistry profile is done, and the patient's total bilirubin is 6.2 mg/dL. What should be done in regard to these results because the normal and abnormal controls are within acceptable limits?
- A. Immediately call the physician to alert him/her to the second abnormal result.
 - B. Immediately send the second result to the patient's floor for charting.
 - C. Repeat the entire second run of patient specimens because there must be an error.
 - D. Perform a delta check and, if warranted, look for possible sources of error.
45. When comparing a potential new test with a comparative method in order to bring a new method into the laboratory, one observes error that is consistently affecting results in one direction. What is this type of error known as?
- A. Systematic error
 - B. Random error
 - C. Constant systematic error
 - D. Proportional systematic error
46. When establishing a reference interval for a new test being introduced into the laboratory, what is the preferred number of subjects that should participate?
- A. 30
 - B. 50
 - C. 75
 - D. 120
47. A small laboratory has collected blood samples from 20 individuals as part of a reference interval study for a new test being introduced into the laboratory. Of the test results, four are outside the reference interval published by the manufacturer. How should you proceed?
- A. Delete the four results and only use the 16 within the range to establish the lab's reference interval.
 - B. Use all 20 results when calculating the $\pm 2 s$ range because outliers are to be expected.
 - C. Run four additional samples and if within the manufacturer's range, add them to the original 16 for statistical analysis.
 - D. Obtain an additional 20 samples for testing, and if two or less are outside the suggested range, then the manufacturer's reference interval can be accepted.

48. Which of the following parameters is *not* needed to determine the predictive value of a positive test?
- Sensitivity
 - Specificity
 - Disease prevalence
 - Precision
49. Which of the following must be known in order to determine the sensitivity of a test?
- True positives and false negatives
 - True negatives and false positives
 - True positives and false positives
 - True negatives and false negatives
50. A new test to assess for the presence of malignancy has been developed. By testing a group of benign individuals, it is determined that 45 of 50 subjects test negative for the new marker. What is the specificity of this new assay?
- 10%
 - 11%
 - 90%
 - 100%
51. Which of the following must be known in order to determine the predictive value of a negative test; that is, the percentage of individuals who test negative and are not diseased?
- True negatives and false negatives
 - True positives and false positives
 - True positives and false negatives
 - True negatives and false positives
52. Which of the following statements is *false* about proficiency-testing programs?
- Participation is mandated by the Centers for Medicare and Medicaid Services under CLIA '88.
 - College of American Pathologists and the American Association of Bioanalysts are two major providers of these programs.
 - Samples of unknown concentrations are periodically sent to labs participating in the program.
 - Acceptable ranges are provided with the samples so labs can determine if it is necessary to repeat the assay.

Laboratory Safety

53. Which of the following is a reactive chemical that has the potential to become shock sensitive if stored for a prolonged period of time?
- Xylene
 - Picric acid
 - Chloroform
 - Phenol
54. A fire extinguisher used in the event of an electrical fire should include which of the following classifications?
- Type A
 - Type B
 - Type C
 - Type D
55. In the National Fire Protection Association identification system, four color-coded, diamond-shaped symbols are arranged to form a larger diamond shape. What type of hazard does the blue diamond identify?
- Flammable
 - Health
 - Reactivity
 - Contact

56. Xylene, ethanol, methanol, and acetone would be in which hazard class?
- Corrosive
 - Flammable
 - Oxidizer
 - Carcinogen
57. Which of the following terms is used to identify a chemical that causes cancer?
- Mutagen
 - Teratogen
 - Carcinogen
 - Reactive
58. A Biosafety Level 2 (BSL-2) laboratory is designed to work with microorganisms that are
- Not associated with disease in healthy adult humans
 - Associated with serious or lethal human disease for which preventative or therapeutic interventions may be available
 - Likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available
 - Associated with human disease that is rarely serious and for which preventative or therapeutic interventions are often available
59. The flash point of a liquid may be defined as the
- Minimum temperature at which self-sustained ignition will occur
 - Maximum vapor pressure at which spontaneous ignition will occur
 - Temperature at which an adequate amount of vapor is produced, forming an ignitable mixture with air at the liquid's surface
 - Temperature that is 10°C greater than the liquid's boiling point
60. A corrosive material was spilled onto the hand of a laboratorian. After diluting the material under running cold water, what should be done next?
- Consult the material safety data sheet.
 - Wipe up the spill with paper towels.
 - Dilute the spill with water and remove it in a biohazard bag.
 - Go to the nearest hospital emergency department.
61. The federal agency charged with the enforcement of safety and health legislation is the
- College of American Pathologists
 - International Air and Transportation Association
 - Joint Commission
 - Occupational Safety and Health Administration
62. The maximum chemical exposure allowable for an employee during one 8-hour day is the
- Flash point
 - HEPA standard
 - Threshold limit value
 - Limit established by the United Nations
63. The air from a chemical fume hood is
- Recirculated through a HEPA filter
 - Totally exhausted from the building
 - Totally exhausted from the building through a HEPA filter
 - Recirculated through a HEPA filter (30%) and 70% is exhausted to the outside
64. The air-handling system for a microbiology laboratory should
- Maintain negative pressure with respect to the administrative areas
 - Maintain positive pressure with respect to the administrative areas
 - Have no particular requirement
 - Have a HEPA filter

65. Precautions such as using a chemical fume hood, wearing rubber gloves, donning a respirator, and cleaning glassware with a strong acid or organic solvent are consistent with working with
- Corrosives
 - Carcinogens
 - Azides
 - Acids
66. A laboratorian, properly dressed in white pants, laboratory coat, and shoes, prepares to leave the laboratory for lunch. In addition to washing his hands, he should
- Put on safety goggles
 - Remove his laboratory coat
 - Wipe the bench with water
 - Remove polyvinyl gloves and place them into labcoat pocket for future use
67. Which of the following may be a potentially hazardous biological situation?
- Handling specimens collected from patients in isolation according to standard precautions
 - Keeping the centrifuge lid closed until the system has stopped completely
 - Discarding sharp objects, including broken glass, in a puncture-proof container
 - Discarding disposable blood collection needles in the patient's wastebasket
68. Which of the following is associated with proper storage of chemicals?
- All chemicals should be stored in alphabetical order for ease of handling.
 - Flammable chemicals should be stored in a chemical fume hood.
 - Large containers of liquid chemicals should be stored on a top shelf to allow easy visibility from below.
 - Volatile solvents should be stored in small amounts in an explosion-proof refrigerator.
69. A laboratorian spills a bottle of concentrated sulfuric acid and slips in the fluid, exposing the lower length of her body to the burning fluid. What would be the most advisable action for a coworker to take?
- Call security.
 - Put the person under the safety shower.
 - Take the injured person to the nearest hospital emergency department.
 - Pour concentrated base on the person to neutralize the acid.
70. A stat procedure requiring a corrosive reagent (organic acid) is requested. To transport this reagent to the work area under the chemical fume hood a laboratorian should
- Employ a rubber carrier with handles
 - Pour an amount near the storage site and transport it
 - Pipette the required volume and carry the pipette to the work area
 - Carry the brown bottle by the loop with one hand under the bottom of the container
71. Which of the following statements pertains to the safe handling of compressed gases?
- Large cylinders should be loosely placed on a hand cart when being transported.
 - Cylinders must be secured to a wall or bench when in use.
 - Cylinders should be stored along with flammable liquids because both are combustible.
 - Large cylinders should be ordered to avoid frequent movement in and out of stock.
72. The major job-related disease hazard in clinical laboratories is
- Tularemia
 - Salmonella
 - Tuberculosis
 - Hepatitis

73. The responsibility to implement and maintain a safety program in a clinical laboratory belongs to the
- A. State public health laboratory
 - B. Chemical hygiene officer
 - C. Laboratory safety officer
 - D. Chief pathologist
74. Based on the chemical properties of azides, which of the following factors has motivated laboratories to monitor their use?
- A. The buildup of salts can lead to explosions.
 - B. They are corrosive to pipes even when diluted.
 - C. They are extremely volatile.
 - D. They are flammable and dangerous near an open flame.
75. A biological safety cabinet that forces HEPA filtered air downward at the front of the cabinet where the laboratorian is working describes a
- A. Class 1 cabinet
 - B. Class 2 cabinet
 - C. Class 3 cabinet
 - D. Class 4 cabinet
76. The phrase “Standard Precautions” refers to a concept of bloodborne disease control that requires all human blood and other potentially infectious materials
- A. Be treated as if known to be infectious for bloodborne pathogens regardless of the perceived “low risk” of a patient population
 - B. Be treated as if it is not infectious unless it is known to be infectious
 - C. Must be handled using a respirator for aerosol exposure
 - D. Need not be treated with caution unless there is a cut on your hand
77. The biological safety cabinet is the single most useful safety device in the microbiology laboratory. How do Class 2A cabinets differ from Class 2B?
- A. Class 2A exhausts HEPA filtered air into the room.
 - B. Class 2B exhausts HEPA filtered air into the room.
 - C. Class 2B cabinets are larger.
 - D. Class 2A cabinets contain gas jets for a Bunsen burner.
78. Chlorine is most often used in the form of sodium hypochlorite (NaOCl), found in household bleach, for a disinfectant. What dilution of household bleach is recommended by the Centers for Disease Control and Prevention to clean up blood spills?
- A. 1:1
 - B. 1:10
 - C. 1:20
 - D. 1:100
79. The clinical hematology laboratory just received a new disinfectant to use in place of the one normally used. Never having used this particular disinfectant before, how should the lab professional proceed?
- A. Use it full strength; you can always be sure if you do this.
 - B. Read the manufacturer’s package insert and prepare the product according to directions.
 - C. Make the concentration 10% higher than the manufacturer’s recommendations.
 - D. Put the new disinfectant under the sink for storage.

80. What written plan of specific measures must laboratories have in place to minimize the risk of exposure to bloodborne pathogens?
- A. Chemical hygiene plan
 - B. Exposure control plan
 - C. Material safety data sheets
 - D. Infection control plan
81. Which of the following information is *not* found on a material safety data sheet?
- A. Health hazard data
 - B. Fire and explosion hazard data
 - C. First aid measures
 - D. Cost of chemical
82. Work is being done with *Mycobacterium tuberculosis* in the microbiology laboratory. It is important that you enter this laboratory while work is being done with positive samples. What is the most important personal protective equipment you should don before entering this laboratory?
- A. Carbon cartridge respirator
 - B. Mask
 - C. Gloves
 - D. N95 HEPA filter respirator
83. When working with chemicals, the selection of your gloves depends on
- A. The chemical hazard and the tasks involved
 - B. How far you must transport the chemical
 - C. Whether or not you use a chemical fume hood
 - D. Whatever is available in the laboratory
84. What is the maximum capacity of a chemical storage safety can used in the laboratory?
- A. 1 gallon
 - B. 5 gallons
 - C. 20 gallons
 - D. 55 gallons

answers **&** **rationales**

General Laboratory Principles

1.

D. Several types of glassware are commonly used in the laboratory, each having its specific purpose. Corex glass is used in the manufacture of centrifuge tubes and thermometers. Pipettes, beakers, and flasks are generally made from Pyrex or Kimax borosilicate glass.

2.

A. The National Institute of Standards and Technology (NIST) and the College of American Pathologists (CAP) state that volumetric pipettes and flasks must be of certified accuracy. Class A glassware meets federal guidelines and fulfills the CAP requirements. All non-class A glassware must be recalibrated periodically by an acceptable verification procedure.

3.

D. Desiccants are drying agents employed to keep some chemicals, thin-layer chromatography plates, and gases used in gas chromatography from combining with water and becoming hydrated. The most effective desiccant is magnesium perchlorate; one of the least hygroscopic is silica gel. Some desiccants can be regenerated

for repeated use by heating them at a high temperature for several hours.

4.

A. Lavender top blood collection tubes contain EDTA as an anticoagulant. The EDTA chelates calcium, which is required for the coagulation cascade. Heparin is the anticoagulant in green top tubes, sodium citrate in blue top tubes, and potassium oxalate in grey top tubes.

5.

A. The *Système Internationale d'Unités* was established to facilitate a uniform system of measurement. SI units may be classified as base, derived, or supplemental units. Base units were established for each of the seven fundamental quantities of measurement: length (meter), mass (kilogram), time (second), electric current (ampere), amount of substance (mole), temperature (kelvin), catalytic amount (katal), and luminous intensity (candela). Derived units are mathematically calculated from more than one base unit.

6.

A. Centrifugal force depends on the mass and on the speed and radius of rotation. Because most materials being centrifuged in the clinical laboratory have specific gravity close to 1.00, only the speed and radius need be considered. The relative centrifugal force (RCF) is calculated by the formula:

$$\text{RCF} = 1.118 \times 10^{-5} \times r \times (\text{rpm})^2$$

where r = radius in centimeters and rpm = the number of revolutions per minute. The RCF is expressed as a number times the force of gravity (or the number $\times g$). The radius is measured from the center of the centrifuge shaft to the inside bottom of the centrifuge cup. The number of revolutions per minute (rpm) is measured by a tachometer. The centrifugal force is not influenced by temperature.

7.

D. The National Institute of Standards and Technology recognizes the use of five specific types of calibrating weights. Analytical balances should be checked monthly and before accurate analytical work for proper calibration using Class S weights. To calibrate the weights themselves, Class M weights are used to check other weights, because their quality is that of a primary standard. Class P weights are allowed greater tolerance levels than Class S-1, with the latter being used for routine analytical work. Class J weights are used for microanalytical work.

8.

A. Serologic pipettes are “to deliver” (TD) types and are not rinsed out. These pipettes are not self-draining but must be blown out to deliver their entire contents, as is indicated by the two etched rings at the top of the pipette. “To contain” (TC) pipettes, such as Sahli pipettes, must be rinsed out to deliver their entire contents.

9.

B. Desiccators provide a dry environment for chemical materials. A shelf is placed on top of the desiccant on which the material to be stored can be set. A heavy glass cover closes the system. An airtight seal is provided by placing stop-cock grease around the ground glass joints between the desiccator and the lid.

10.

D. In an angle-head centrifuge, the cups are rigidly supported in the head at a fixed angle to the shaft, and they are fully enclosed within the head. In a horizontal-head centrifuge, the cups hang down in a vertical position when the centrifuge is at rest and swing out to a horizontal position when the centrifuge is rotating. Because cups in the angle-head centrifuge are enclosed in a head specifically designed to reduce wind resistance, there is less air friction and, consequently, less of an increase in sample temperature during centrifugation. Because of the reduced wind resistance, angle-head centrifuges can provide a force of over $9000 \times g$, whereas horizontal-head centrifuges provide about $1650 \times g$.

11.

C. Reagent grade water should be used when a high degree of accuracy is desired, as in quantitative chemistry assays and in the preparation of standards and buffers. Reagent grade water requires deionization through acidic and basic ion-exchange columns, removal of organic materials by activated charcoal adsorption, and semi-permeable membrane filtration for the removal of microorganisms and other particulate material. Special reagent water is for specific uses and, depending upon the use, may need to be sterilized.

12.

A. Centrifugal force may be determined by knowing the mass of the solution and the speed and radius of the centrifuge. With aqueous solutions having a specific gravity near 1.0, the specific mass need not be known. To determine the speed, use either a strobe light, positioned over the revolving centrifuge head, or a tachometer to establish the revolutions per minute (rpm).

13.

D. An electronic balance is a single-pan balance that uses an electromagnetic force to counterbalance the load placed on the pan. These balances are top-loading in design and permit weighings to be made quickly. The Mettler Instrument Corporation makes a representative electronic balance.

14.

D. Speckled top tubes, without an anticoagulant, contain a separation gel and are referred to as serum separator tubes. Plasma separator tubes contain the separation gel and an anticoagulant, such as lithium heparin; they may have a speckled or solid colored top representing the anticoagulant. During centrifugation, the gel forms a barrier between the blood cells and the serum or plasma. The gel barrier helps to maintain the integrity of the analytes in the liquid portion, and it facilitates automatic sampling or removal of the serum or plasma from the tube.

15.

A. To prevent anticoagulant carryover and contamination when using a needle and needle holder for blood collection, evacuated blood collection tubes should be collected in a proper order. Sterile specimens should be drawn first to minimize risk of contamination for blood cultures. After sterile collections, the following order should be used: light blue top, plain red

top or serum separator tube, green top (heparin or heparin PST), and lavender top.

Laboratory Quality Assessment

16.

C. The arithmetic mean of a set of numbers is obtained by adding all the numbers in the set and dividing the sum by the number of values in that set. It is a precise way of expressing what is often called the average. It is not to be confused with the mode, which is the value that occurs most frequently in the set. The geometric mean is the antilogarithm of the sum of the logarithms of all the values divided by the number of values. The median is the middle value in a set of numbers that are arranged according to their magnitude.

17.

B. The coefficient of variation is calculated from the formula:

$$CV = \frac{s}{\bar{x}} \times 100\%$$

where CV = coefficient of variation, s = 1 standard deviation, and \bar{x} = mean. Given that the mean = 89 mg/dL and $2s$ = 14 mg/dL,

$$CV = \frac{7}{89} \times 100\% = 7.86\%$$

Because there are only two significant figures in each of the given numbers, there can be only two figures in the answer. Therefore, when rounded to the nearest tenth, the answer is 7.9, not 7.8.

18.

A. Any analytical result has some degree of uncertainty because of unavoidable random errors in the procedure. A Levey-Jennings quality control chart is a graphic representation of the acceptable limits of variation in the results of an analytical method. To prepare such a chart, it is first necessary to obtain a large-enough batch of normal and abnormal pooled serum to last for a minimum of 12 months. Analyses of aliquots of the pools are done in duplicate over a period of 20 days, preferably by all workers who will subsequently be using the controls. The data thus collected are statistically analyzed to determine the mean and standard deviation. Any results falling above or below the mean $\pm 3 s$ are discarded. The mean and standard deviation are then recalculated. The acceptable range is assigned, usually the mean $\pm 2 s$. The data thus developed are used to prepare the Levey-Jennings quality control chart. A similar protocol may also be followed when setting up standard deviation parameters for new lots of assayed control materials.

19.

B. The purpose of a quality control chart is to facilitate the identification of analytical problems that are not otherwise apparent. A quality control program must include clearly written instructions for the steps that are to be taken when a control serum value is out of control. These instructions must be used whenever a set of test results is out of the established control limits. Usually the procedures will include a visual inspection of the equipment, reagents, and instruments used, and a check of calculations. The next step might be to rerun the batch of tests with a fresh aliquot of control serum. Additional steps to take include preparing newly reconstituted controls, recalibrating the instrument, and using a fresh bottle of reagent. Out-of-control results should never be reported to the physician.

20.

A. When assessing daily, internal quality control, the Westgard multirule procedure aids in interpretation of control data. A chart similar to the Levey-Jennings chart is constructed with control limits drawn at the mean as well as $\pm 1 s$, $\pm 2 s$, $\pm 3 s$, and even $\pm 4 s$. The Westgard multirules are then applied to the graphical representation, giving a more structured approach to data interpretation.

21.

C. On a quality control chart, when the control values change abruptly and on several consecutive days are consistently on one side of the mean, although within the $\pm 2 s$ limits, this is called a shift. An upward shift could be produced by changing to a new standard (calibrator) that was prepared in error at a lower concentration than specified. A downward shift could be caused by the use of too concentrated a standard (calibrator) than what is specified. A gradual change observed over the course of several days is called a trend. It may be upward or downward. Its presence suggests gradual deterioration of one of the reagents or instrument components.

22.

C. If the range of acceptable values for a quality control material is based on the $\pm 2 s$ intervals on either side of the expected mean value for the control, then about 19 of every 20 values obtained for the control are expected to fall within the acceptable range. Conversely, about 1 of every 20 values obtained are expected to fall outside the $\pm 2 s$ range with about 1 of every 40 values above the upper acceptable limit and the same number below the acceptable limit. For example, if a digoxin control is established to have an acceptable range from 2.0 to 2.6 ng/mL, about 1 value in 40 would be expected above 2.6 ng/mL.

23.

A. A Youden plot is a type of quality control chart that is used to compare results obtained on a high and low control serum by several different laboratories. It is particularly useful for interlaboratory quality control programs. The Youden plot displays the results of the analyses by plotting the mean values for one specimen on the ordinate and the other specimen on the abscissa. It is desirable for a laboratory to have its point fall at the center of the plot.

24.

C. The choice of appropriate concentrations for control materials is important in implementing a quality control program. The concentrations chosen should be sensitive to assay variability in the clinically significant region of the particular compound being measured. For instance, the therapeutic range for the drug gentamicin in many laboratories is a trough level that is less than 2 $\mu\text{g/mL}$ and a peak level of 5–8 $\mu\text{g/mL}$. Control levels of 1.5 and 6 $\mu\text{g/mL}$ would appropriately monitor both trough and peak regions of the standard curve. If both controls are above 5 or below 4 $\mu\text{g/mL}$, only one region of the curve would be monitored.

25.

A. Scheduling of staff is not involved in quality assessment programs. Quality assessment in a broad sense includes monitoring every aspect of laboratory work from collection and identification of specimens to delivery of valid results to the physician. Laboratory protocol should include procedures that ensure that the correct specimen is collected and that the specimen is correctly labeled. The quality of the laboratory supplies, including the reagent grade water (CLRW) and reagents, should meet the specifications of the appropriate professional or governmental agency (i.e., the College of American Pathologists, the American Chemical Society, or the National Institute of Standards and Technology). The

accuracy and precision of the analyses should be ensured through the use of appropriate standards (calibrators) and controls. Also, quality control charts that define the acceptable range of results for control specimens and participation in external proficiency testing programs are part of any quality assessment program. Because most analyses are done with instruments, the performance of the instruments must be monitored. Establishment of a regularly scheduled preventive maintenance program provides for optimum performance of the instruments as well as continuous monitoring of their performance.

26.

D. On a quality control chart the acceptable range generally encompasses the mean $\pm 2s$. The control values should occur randomly in this area, falling to both sides of the mean. If more than five successive plots occur at a constant level in one area (e.g., near the $\pm 2s$ line), an out-of-control situation should be considered. One plot falling outside the mean $\pm 2s$ in 20 successive days is expected statistically. Although one should be alert to the possibility of a potential problem, it does not necessarily imply an out-of-control situation. However, the occurrence of a value outside the area of $\pm 3s$ would require corrective action.

27.

C. When a quality control chart shows a sudden shift in daily values, there are several possible causes. Use of a new batch of reagents or reference standards (calibrators) that have been improperly made can cause such a shift. Another cause for a sudden shift in daily values might be a change in one of the components of the instrument, such as a new lamp in a spectrophotometer. Whenever an instrument component is changed, the instrument must be recalibrated. A change in operating personnel should not cause any change in quality control values.

28.

D. The standard deviation reflects how much the data values vary around the mean. The mean is the arithmetic average of the data and is a measure of the location of the distribution. The median describes the middle value; half of the observations are greater than the median, half are less than the median. The mode is the most frequently obtained value. The coefficient of variation expresses random variation of analytical methods in units independent of methodology, because it is a percentage comparison of the standard deviation divided by the mean.

29.

B. Specificity is the percentage of individuals without the specific disease who are correctly identified or predicted by the test as not having the disease. The sensitivity of a test is the percentage of individuals with a specific disease who are correctly identified or predicted by the test as having the disease. The positive predictive value is the percentage of people who tested positive with the test who have the disease, and the negative predictive value is the percentage of people who tested negative with the test who do not have the disease.

30.

A. Random errors are deviations from the true value caused by unavoidable errors inherent in laboratory measurements. The standard error of the mean is a statistical concept reflecting sampling variation. It is the standard deviation of the entire population. Parametric statistics refer to a Gaussian (normal) distribution of data. Nonparametric statistics are more general and require no assumptions.

31.

D. To determine the standard deviation, compute the difference between each value and the mean ($x - \bar{x}$), square the differences, and add

all the squared differences $[\sum(x - \bar{x})^2]$. Then divide the sum by one less than the number of values ($n - 1$) and take the square root. The standard deviation is the estimated random error.

32.

C. The accuracy of an analytical result is the closeness with which the measured value agrees with the true value. Precision is reproducibility. Accuracy and precision are independent, but it is the goal of the clinical laboratory to design methods that are both precise and accurate.

33.

C. The normal distribution is a symmetric distribution about the mean. In a normal distribution, 95.45% of the values will be within an area enclosed by the mean $\pm 2s$ and approximately 5% will normally fall outside; 68.26% will lie within $\pm 1s$; 99.74% will lie within $\pm 3s$. The $\pm 2s$ (95.45%) interval forms the basis of statistical quality control in the laboratory.

34.

C. Variance is one way in which members of a group are dispersed about the mean. It is a square of the standard deviation (s^2). Both standard deviation and variance are measures that describe how observed values vary.

35.

A. The reliability of an analytical procedure is its ability to maintain accuracy and precision over an extended period of time during which supplies, equipment, and personnel in the laboratory may change. It is often used interchangeably with the term "consistency." It is the goal of every clinical laboratory to produce reliable results.

36.

B. The variance is the measure of dispersion, which is the square of the standard deviation. To determine the variance, find the difference between each value and the mean, square this difference, add the differences, and divide by one less than the number of values. The variance reflects scattering about the mean.

37.

C. A control is a specimen of known concentration with physical and chemical properties closely resembling the test specimen. The control is generally composed of the same matrix as the sample. The concentrations of analytes in different control materials should be in the normal and abnormal ranges. Both primary standards and calibrators are used to calculate the concentration of specimens being analyzed. Calibrators are considered secondary standards and must meet specific criteria as outlined by the National Institute of Standards and Technology. These criteria need not be met for controls and thus their uses cannot be interchanged.

38.

D. Maintaining quality assessment includes control of preanalytical, analytical, and post-analytical factors. One variable to assess is turnaround time. It is the total amount of time required to procure the specimen, prepare the specimen, run the test, and relate the results. The supervisor should refer to the turnaround time of the stated procedure and relate to the techs the need to work within the stated limits.

39.

D. The College of American Pathologists comprehensive survey involves thousands of participating clinical chemistry laboratories. This survey and others have been established to provide independent validation of quality control

programs. A CAP survey provides unknown samples for analysis. The program, when properly used, gives valid estimation of the inherent accuracy of a system. CLSI develops laboratory standards to improve the quality of medical care. ASCLS and ASCP are organizations to which medical laboratory personnel may apply for professional membership.

40.

B. Whenever one needs to review the details of a procedure, one should review the procedure manual and notify the supervisor so that guidance can be received. The procedure manual is one way in which a laboratory can document analytical protocols. This leads to consistency in test results regardless of which person is performing the analysis.

41.

C. When checking control results that fall outside acceptable limits, one can apply the Westgard multirule procedure, specifically 1_{3s} . Anytime only one control is used and it exceeds the mean $\pm 3s$, you must reject the test run. You should check out the instrument and reagent system to locate the problem if possible. A new control along with the patient specimens should be analyzed. No results should be reported until the control is within the limits of $\pm 2s$ from the mean.

42.

C. Westgard multirule 2_{2s} describes an out-of-control situation where two consecutive data points fall outside the same mean $+2s$ or fall outside the same mean $-2s$. This is an example of systematic error. The test run would be rejected, and all samples would need to be retested.

43.

D. When one control point exceeds the mean by $+2s$ and a second control point exceeds the mean by $-2s$, the R_{4s} multirule will apply. In this case the out-of-control problem is most likely due to random error. The test run would be rejected, and all samples would need to be retested.

44.

D. When the same test is ordered on a patient more than once, a delta check can be performed to compare consecutive test results. Bilirubin results obtained on two consecutive days on an adult should not vary by more than 50%. If the results vary by greater than 50%, it is most likely that an error has occurred or an acute change has taken place. One of the first things to check is proper identification of the patient's specimen. As part of a quality assessment program, one should also check patient results based on the clinical correlation of laboratory test results.

45.

A. When comparing a potential new test with a comparative method in order to bring a new method into the laboratory, linear regression analysis should be performed using the results of the two methods. By calculating the slope and y intercept, the presence of systematic error can be identified. Unlike random error that is due to chance and can occur in either direction, systematic error consistently affects results in only one direction. Specific types of systematic error are termed constant and proportional, but the stated question did not give sufficient information to differentiate between the types.

46.

D. Although some laboratories may use the reference interval recommended by the manufacturer or ranges published in medical books, it is preferred that laboratories establish their own limits. When subjects are not easily available, a

laboratory should use at least 20 individuals to verify a published range. Whenever possible, a minimum of 120 subjects with representatives from each age and sex group should be included in the reference interval study.

47.

D. When the population served by a laboratory is similar to that described by a manufacturer, then the reference interval published by the manufacturer can be adopted provided the laboratory successfully completes a small study. Such a study need only include 20 individuals. If two or less subjects tested have test values that fall outside the suggested range, then the manufacturer's reference interval can be used. If three or more subjects have test values that fall outside the range, then an additional 20 subjects need to be tested. Provided that two or less are outside the range on this second attempt, then the manufacturer's reference interval can be accepted. In the event that this second attempt fails, the laboratory should assess what differences there may be between their population and that of the manufacturer. If differences cannot be determined, then a complete reference interval study using 120 subjects should be completed by the laboratory.

48.

D. To determine the percent of individuals with a positive test result who actually have the disease, the predictive value of a positive test is calculated. The sensitivity, specificity, and disease prevalence must be known to calculate the predictive value. Sensitivity in this case refers to the percent of individuals having the disease who test positively. Specificity refers to the percent of individuals who do not have the disease and test negatively. Precision is not a component of the formula.

49.

A. The *sensitivity* of a test is the percentage of individuals with a specific disease that are correctly identified or predicted by the test as having the disease. To determine the sensitivity of an assay, the true positives, represented by the number of individuals correctly identified by the test as having the disease, and the false negatives, represented by the number of diseased individuals not correctly identified by the test, must be established for the assay in question. The formula for determining sensitivity follows, where TP = true positives and FN = false negatives.

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100$$

50.

C. The *specificity* of a test is the percentage of individuals without the specific disease that are correctly identified or predicted by the test as not having the disease. To determine the specificity of an assay, the true negatives, represented by the number of individuals correctly identified by the test as not having the disease, and the false positives, represented by the number of nondiseased individuals not correctly identified by the test, must be established for the assay in question. The formula for determining specificity follows, where TN = true negatives and FP = false positives.

$$\begin{aligned} \text{Specificity} &= \frac{\text{TN}}{\text{FP} + \text{TN}} \times 100 \\ &= \frac{45}{5 + 45} \times 100 \\ &= \frac{45}{50} \times 100 \\ \text{Specificity} &= 90\% \end{aligned}$$

51.

A. The predictive value of a test utilizes the parameters of test sensitivity and specificity as well as disease prevalence. The predictive value of a negative test (i.e., the percentage of individuals

who test negative and are not diseased) may be determined by knowing the number of true negatives and false negatives. The formula for determining the predictive value of a negative test (PV⁻) follows, where TN = true negatives and FN = false negatives.

$$\text{PV}^- = \frac{\text{TN}}{\text{TN} + \text{FN}} \times 100$$

52.

D. A proficiency testing program is part of external quality control that aids a lab in assessing the quality of its testing methods. Samples of unknown concentrations are purchased through a recognized professional agency such as the College of American Pathologists or the American Association of Bioanalysts. Samples are periodically sent to labs participating in the program. Following analysis of the samples, the lab sends its results to the agency for review. If significant problems are detected, the laboratory needs to take corrective action. Participation is mandated by the Centers for Medicare and Medicaid Services under CLIA '88.

Laboratory Safety

53.

B. Picric acid has the potential to form peroxides if stored for a long period of time and not used. If this happens, the bottle can become shock sensitive with the potential to explode if knocked. All other chemicals listed do not pose this risk.

54.

C. A fire extinguisher is classified and labeled for the type of fire on which it should be used. An ABC fire extinguisher is commonly found in laboratories. Type A extinguishers are used on fires of ordinary combustibles such as paper, cloth, wood, rubber, and plastics. Type B extinguishers are used on fires of flammable liquids including oils, gasoline, and solvents. Type C extinguishers are used on electrical equipment fires. Type D extinguishers are used on fires involving combustible metals (e.g., magnesium, sodium).

55.

B. The National Fire Protection Association developed the 704-M Identification System to provide common, recognizable warning signals for chemical hazards. The system consists of four color-coded, diamond-shaped symbols arranged to form a larger diamond shape. The blue diamond symbol located to the left identifies potential health hazards. The diamond symbol located at the top of the larger diamond is color-coded red, indicating a flammability hazard. The yellow diamond symbol to the right represents reactivity-stability hazards. The white diamond symbol located at the bottom provides information on special precautions. Contained within each color-coded diamond is a number ranging from 0 to 4, indicating the severity of the respective hazard (0 = none and 4 = extreme). A number of chemical manufacturers have adopted this warning system for their labels.

56.

B. All hazardous chemicals in the workplace must be identified and clearly marked with a National Fire Protection Association label. All the chemicals listed are flammable. Corrosive chemicals are harmful to mucous membranes, skin, eyes, or tissues.

57.

C. A carcinogen is defined as a substance or agent producing or inciting cancer. Mutagens cause changes in DNA. Teratogens cause birth defects.

58.

D. The National Institutes of Health guidelines describe Biosafety Level 2 laboratories as those laboratories that work with microorganisms associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. Biosafety Level 1 laboratories handle agents that have no known potential for infecting healthy people.

Biosafety Level 3 is recommended for materials that may contain viruses not normally encountered in a clinical laboratory.

59.

C. Both flammable and combustible liquids are commonly used in the laboratory. These two categories are differentiated on the basis of their flash points—that is, the temperature at which a liquid forms an adequate amount of vapor to produce an ignitable mixture with the air at the liquid's surface. The flash point of flammables is designated as less than 100°F and that of combustibles as greater than 100°F.

60.

A. Manufacturers of chemicals, reagents, and kits provide material safety data sheets (MSDSs) for all products. These sheets must be available to all laboratorians in case of emergency. When an individual goes to the emergency department, he or she should have the MSDS to give to the physician in order to get prompt, correct treatment. A laboratory professional must be confident to report any accident and take appropriate measures to clean it up.

61.

D. The Occupational Safety and Health Administration is the federal agency charged with the enforcement of safety and health legislation. The College of American Pathologists and The Joint Commission are private organizations that issue standards and offer accreditation. The International Air and Transportation Association develops regulations for the shipment of hazardous materials.

62.

C. The threshold limit value (TLV) is the exposure allowable for an employee during one 8-hour day. The TLV will be listed in the material safety data sheet for each chemical. More toxic chemicals will have a smaller TLV.

63.

B. A chemical fume hood is an engineering control to provide protection from chemicals. Correct airflow is critical in containing fumes. All air in a chemical fume hood is exhausted out of the building. Chemicals should not be used in a biological safety cabinet unless a Class 2B cabinet can be used, in which case all the air is also exhausted to the outside but through a HEPA filter.

64.

A. Laboratory areas should maintain negative pressure with respect to the administrative areas to prevent toxic or pathogenic materials used in laboratory work areas from escaping and injuring humans or contaminating the environment. The amount of air provided to the negative pressure laboratory should be equal to 85% of the air exhausted from the area. Positive pressure is maintained in the office areas.

65.

B. Some of the precautions that should be followed when working with carcinogenic chemicals include performing the procedure in a chemical fume hood, wearing rubber gloves and proper protective clothing, and wearing a respirator when working with organic vapors and dust-producing materials. If possible, use disposable glassware. All other glassware should be washed with a strong acid before being processed in the general wash cycle.

66.

B. Safe practices in the laboratory are essential to the well-being of all employees. Each laboratorian should disinfect his/her work area daily. Pens and pencils placed on laboratory bench tops may be contaminated and should never be placed near one's mouth. Laboratory coats should never be worn in the cafeteria because they may be contaminated. Laboratory personnel should never smoke, eat, or drink in the laboratory,

nor should food be placed in a refrigerator used for storage of reagents or biologic specimens. Cosmetics should not be applied in the laboratory because of potential contamination. Personnel should always wash their hands before leaving the laboratory, discarding their used gloves in a biohazard receptacle.

67.

D. It should be remembered that all body fluids from patients are potentially hazardous to one's health. Specimens collected from infectious patients in isolation should be handled according to standard precautions. Blood specimens need to be centrifuged, and inhalation of aerosols is prevented by never raising centrifuge lids prematurely. All sharp objects, including broken glass and needles, should be disposed of in a puncture-proof container. Blood collection needles should never be discarded in a wastebasket in a patient's room, because housekeeping personnel or others may easily be injured and infected.

68.

D. Chemicals should not be stored in alphabetical order because some chemicals are incompatible with others and will react adversely. Large containers of chemicals should always be stored on a shelf as close to the floor as possible to avoid severe injury in the event of breakage. Flammable chemicals should be stored in a fire-safety cabinet. Although flammables should be used in a chemical fume hood, the hood is not a proper storage area.

69.

B. Emergency showers must be available to anyone working with corrosive materials. The victim should be removed from the area as rapidly as possible and showered with water. No attempt should be made to neutralize the acid on the person's skin.

70.

A. Bottles of chemicals and solutions should be handled carefully. Chemical containers made of glass should be transported in rubber or plastic holders that will protect them from breakage. In the event of breakage, the plastic holders will contain the spill.

71.

B. Compressed gas cylinders should be stored in a vertical position in a ventilated, fire-resistant location. Gas cylinders must never be stored in the same area as flammable liquids, because both are highly combustible. Because of their shape, gas cylinders may easily fall, causing the regulator valve to rupture. To prevent such an occurrence, cylinders must always be fastened when stored, transported, and used in the laboratory.

72.

D. Viral hepatitis is the major job-related disease hazard in all clinical laboratories. All laboratorians who handle blood or body fluids are at risk. The modes of transmission include ingestion and injection. Thus, it is crucial that the laboratorian follows proper safety practices at all times.

73.

C. The responsibility to implement and maintain a safety program belongs to the laboratory safety officer. The responsibility of the chemical hygiene officer is to provide technical guidance in the development and implementation of the chemical hygiene plan. Accreditation agencies typically require laboratories to have both a safety officer and a chemical hygiene officer.

74.

A. Although now considered a carcinogen, sodium azide has been used as a preservative in some laboratory reagents. When disposal of this reagent is made in the sewer, a build-up of copper and iron salts of azide may occur. These

metallic salts are explosive, especially when subjected to mechanical shock.

75.

B. The single most useful safety device used in a clinical microbiology laboratory is the Class 2 biological safety cabinet. This engineering device is designed with inward airflow at a velocity to protect personnel. In addition, it is constructed with HEPA-filtered vertical laminar flow for protection of laboratorians.

76.

A. In 1987, the Centers for Disease Control and Prevention (CDC) established guidelines for universal precautions. These guidelines were established to lower the risk of hepatitis B virus and human immunodeficiency virus transmission in clinical laboratories and blood banks. In 1996, the CDC published new guidelines, called standard precautions, for isolation precautions in hospitals. Standard precautions (updated in 2007) synthesize the major features of body substance isolation and universal precautions to prevent transmission of a variety of organisms.

77.

A. Class 2A biological cabinets force HEPA-filtered air downward at the front of the cabinet where the laboratorian is working. This provides a barrier between the worker and the infectious material. Class 2A biological cabinets exhaust HEPA-filtered air into the building, whereas Class 2B cabinets exhaust air out of the building.

78.

B. Halogens, especially chlorine and iodine, are frequently used as disinfectants. Chlorine is most often used in the form of sodium hypochlorite (NaOCl), the compound known as household bleach. The Centers for Disease Control and Prevention recommends that counter tops be cleaned following blood spills with a 1:10 dilution of bleach.

79.

B. The most important point to remember when working with biocides or disinfectants is to prepare a working solution of the compound exactly according to the manufacturer's package insert. Many people think they will get a stronger product if they use a more concentrated dilution. The ratio of water to active ingredient may be critical, and if sufficient water is not added, the free chemical for surface disinfection may not be released.

80.

B. Each employer having an employee with occupational exposure to human blood or any other infectious materials including bloodborne pathogens must establish a written exposure control plan designed to eliminate or minimize employee exposure. The plan identifies tasks that are hazardous and promotes employee safety. The plan incorporates education, proper disposal of hazardous waste, engineering controls, use of personal protective equipment, and a post-exposure plan.

81.

D. Material safety data sheets (MSDSs) will specifically include chemical identity as it appears on the label, chemical name and common name, physical and chemical characteristics, signs and symptoms of exposure, routes of entry, exposure limits, carcinogenic potential, safe handling procedures, spill cleanup procedures, and emergency first-aid. MSDSs are provided by the manufacturers for every chemical. MSDSs contain information on the nature of the chemical, precautions if spilled, and disposal recommendations.

82.

D. *Mycobacterium tuberculosis* is spread by the aerosol route. The risk of inhalation of infectious materials can occur in the laboratory environment and poses a significant potential health hazard to the employees. The proper personal protective equipment is extremely important when working with particular infectious materials. The N95 HEPA filter respirator is a high-energy particulate air filter and is used for microorganisms spread via the aerosol route.

83.

A. Hands are more likely to contact chemicals than any other part of the body. Gloves made of appropriate materials can effectively protect the hands from exposure if they are worn during routine handling of chemicals. Selection of protective gloves is based on chemical hazard and the tasks involved. The glove fabric must have an acceptable slow breakthrough time and permeation rate for the chemical of interest.

84.

B. A safety can is used to store used chemicals until the material is removed from the laboratory. An approved container has a capacity of no more than 5 gallons. A safety can has a spring-closing lid and spout cover, and it is designed to safely relieve pressure buildup within the container.

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